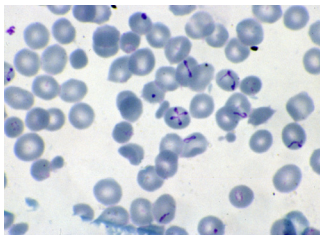

11

Babesiosis
(*Piroplasmosis*)

George D. Imes, Ronald C. Neafie, and Francis M. Chiricosta

Definition

Babesiosis is a malaria-like, zoonotic disease transmitted by ticks and caused by protozoa of the genus *Babesia*, which invade and destroy erythrocytes.

Synonyms

Babesia has been known by other genus names, including *Piroplasma*, *Nuttallia*, *Microbabesia*, *Babesiella*, and *Gonderia*. Babesiosis has been called tick fever, Texas tick fever, Texas cattle fever, red water, bloody murrain, splenic fever, and biliary fever. Because all *Babesia* species are piroplasms, a more inclusive term for human infections caused by these organisms would be piroplasmosis. Development and refinement of methods for identifying and classifying organisms has resulted in some *Babesia* and *Babesia*-like organisms being reclassified to other genera, such as *Theileria*.[1,2] (Fig 11.1)

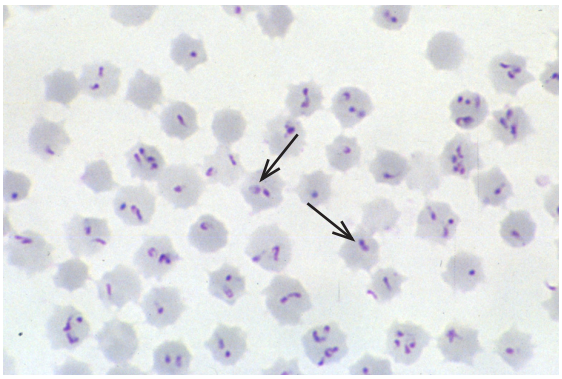

Figure 11.1
Theileria parva from a cow in Africa. Note numerous small pear or tear-drop shaped piriforms (arrows) in erythrocytes. Giemsa. Original magnification x330

General Considerations

Babès discovered the organism in Romania in 1888 while studying cattle dying with fever and hemoglobinuria. Believing the organism was a bacterium, be called it *Haematococcus bovis*.[3] In 1889 Smith and Kilborne recognized a protozoon as the cause of Texas cattle fever and called it *Pyrosoma bigeminum* (*Babesia bigemina*) (Fig 11.2). In 1893 they established the role of the tick *Boophilus annulatus*, the first proven arthropod vector of an infectious disease, in the transmission of Texas cattIe fever.[4] They also outlined the transovarial transmission of infection by the female tick to her offspring, an essential element that explained the unusual epizootiology of Texas cattle fever.

In 1904, Wilson and Chowning observed pirosomes that were undoubtedly *Babesia* in the blood of patients who had Rocky Mountain spotted fever in Montana.[5] The first human infection was documented in 1957 in a splenectomized Yugoslavian from an area where cattle were infected with *Babesia bovis* (Fig 11.3).[3] In June of 1966, a 46-year-old splenectomized resident of San Francisco became ill and later diagnosed with babesiosis. This Californian frequented sparsely populated areas. The species of the *Babesia* was not determined, but was thought to be from a wild animal,

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
JUN 2011		**00-00-2011 to 00-00-2011**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Babesiosis (Piroplasmosis)	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Armed Forces Institute of Pathology,Washington,DC,20306**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES
See also ADA545141. Chapter 11 from e-book, Topics on the Pathology of Protozoan and Invasive Arthropod Diseases.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**13**	

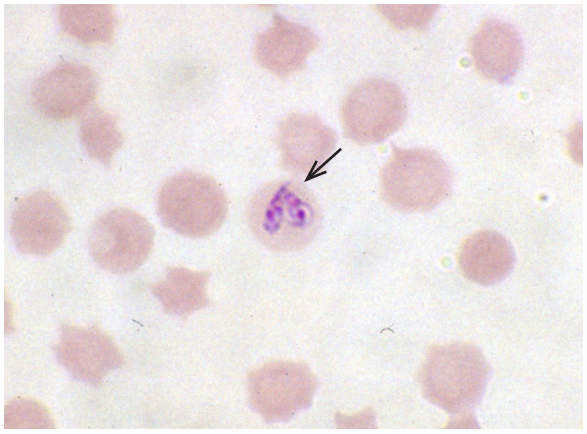

Figure 11.2
Thin blood film showing 2 piriform *Babesia bigemina*, a parasite of cattle, attached at their ends (arrow). Giemsa. Original magnification x330

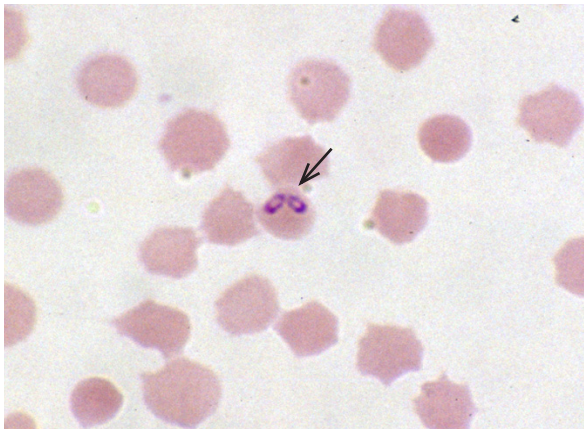

Figure 11.3
Thin blood film of *Babesia bovis* (arrow), a parasite of cattle. Note typical paired trophozoites resulting from binary fission. Giemsa. Original magnification x330

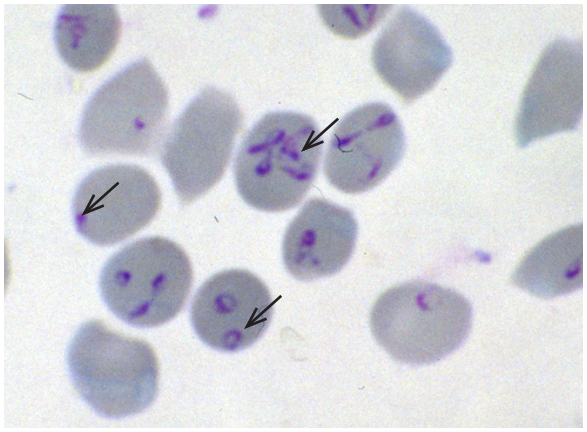

Figure 11.4
Thin blood of *Babesia divergens* from human patient, Note erythrocytes infected with multiple parasites in ring forms, divergent forms (forms diverging at a wide angle of up to 180°), piriform, and chromatin dots (arrows). Giemsa. Original magnification x330

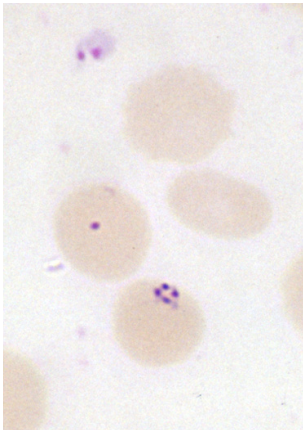

Figure 11.5
Thin blood of *Babesia microti* in patient from Fire Island, New York. Note tetrad formation following schizogony. Giemsa. Original magnification x330

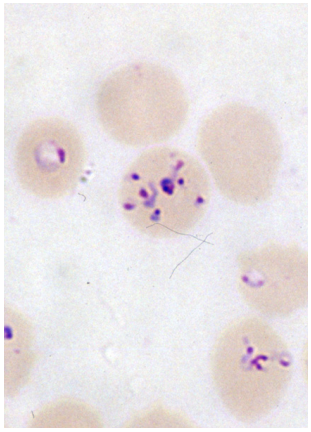

Figure 11.6
Babesia microti from patient described in Figure 11.5. Note ring forms and pleomorphism. One erythrocyte contains at least 8 parasites.Giemsa. Original magnification x330

most likely a small rodent.[6] In 1968, a third case of human babesiosis was reported in another splenectomized patient.[7] This fisherman from Northern Ireland fell ill August 29, 1967 and died September 5. In this patient the infectious agent was called *Babesia divergens*, another cattle *Babesia* (Fig 11.4). In 1969, human babesiosis developed in a non-splenectomized patient from Nantucket Island. Significantly, this was the first time *Babesia microti* was implicated as a cause of human babesiosis (Figs 11.5 and 11.6).[8]

The first reported transmission by blood transfusion was in 1979.[9] Transfusion transmitted babesiosis is recognized as an increasing problem even outside endemic areas.[10-15] More than 100 transfusion-transmitted cases have been reported, most in the US, but also in Europe and Japan.[16-18] At least 11 deaths following transfussion have been reported in the US.

Approximately 100 species of *Babesia* are currently known and new species are being discovered. *Babesia* infect a wide variety of wild and domestic animals, including cattle,[19] sheep, goats, wild ruminants, horses, donkeys, dogs, cats, swine, raccoons, skunks, fowl, monkeys, and wild rodents. Despite this wide distribution among animals, human infections are not common, probably due to the characteristic host specificity of *Babesia*. Although first documented in humans only about half a century ago, the disease has likely afflicted humans for much longer. Humans are susceptible to infection with: 1) *B. microti*, which infects several wild rodent species and is the cause of most human infections reported in the United States; 2) *B. divergens*, which primarily infects cattle in continental Europe,

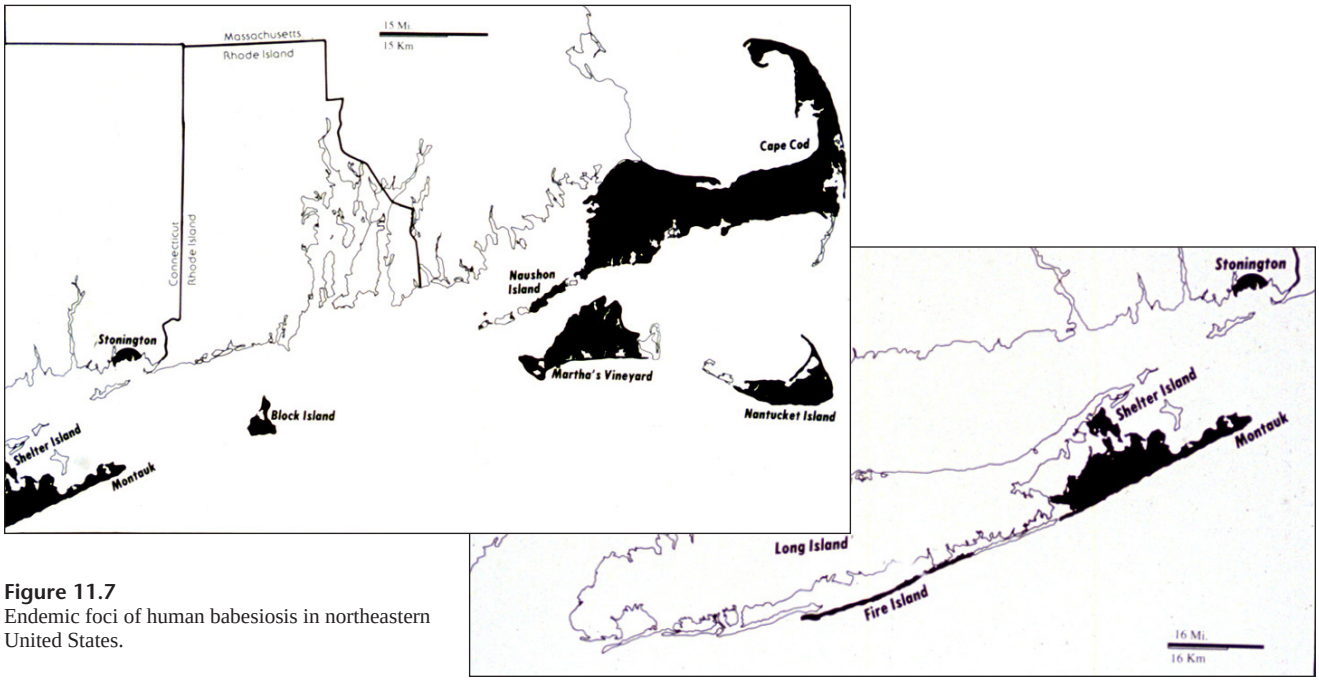

Figure 11.7
Endemic foci of human babesiosis in northeastern United States.

the United Kingdom, and Ireland, but also infects splenectomized humans and some rodent species; and 3) novel *Babesia*-like organisms detected by microscopy but with genetic, biologic, and serologic differences from known species. Examples include parasites called WA1, CA1, MO1 and EU1. WA1 and CA1 were the etiologic agents in several human infections in Washington State and California; MO1 has appeared only in Missouri; EU1 was obtained from patients in Italy and in Austria.

Epidemiology

Incidence of clinical infection with *Babesia* organisms is highly variable from location-to-location. Infection depends on many factors: 1) an animal reservoir; 2) a *Babesia* to which humans are susceptible, or a human host who is asplenic or otherwise immunocompromised; 3) a genus of *Ixodidae* (hard bodied tick) which can transmit the parasite; and 4) a suitable tick habitat. Finally, humans must put themselves at risk by frequenting a tick habitat in an endemic area in the months when infective stages of ticks are feeding. Transfusion transmission can occur anywhere and outside of tick-feeding season, because blood for transfusion can be collected in a different region of the country and asymptomatic donors may carry the parasite. In the US, as of January 2011, babesiosis is a nationally-reportable disease.[20] The systematic collection of these data nationwide should help improve knowledge of the geographic distribution of babesiosis and possibly the spectrum of disease.

Endemic areas in the United States have included islands off the southeastern coast of New England, including Long Island, New York (Fig 11.7),[21,22] and areas of some eastern and upper Midwestern states.[23] Increasing prevalence of tick-borne babesiosis has spread to Rhode Island, New Jersey, and Maryland.[24-26] In the western United States, babesiosis was reported twice prior to the 1990s; both cases involved asplenic patients from California. The protozoon in these infections was a small, unidentified *Babesia* that formed tetrads within erythrocytes.[6,27] The species in these cases was not determined. From 1991 to 1994, six additional cases of babesiosis were diagnosed; four in California[28] and two in Washington State.[11,29,30] One of the cases in Washington was acquired via transfusion. The morphology of these parasites was similar to the earlier reports from California, and to *B. microti*.[31] One of the California infections was suspected to be *Babesia gibsoni* (Fig 11.8), a small *Babesia* of dogs, based on serologic findings.[32] Sequence analysis of the gene for ribosomal RNA showed these and the parasite from the first Washington case to be closely related to *B. gibsoni*.[30,31] The patients who survived their acute illness developed elevated titers of antibody to *B. gibsoni* and to WA1 antigens from the index case in Washington. They did not have cross reacting antibody to *B. microti*.[28,33] These organisms had been termed WA1 and CA1 (the numeral indicating the index case, and applied to the parasite type in general.) The WA1 parasite was later named *Babesia duncani*.[31] Another transfusion-transmitted case of *B. duncani* (WA1) occurred in California in 2000.[34] These seven reported symptomatic infections are insufficient to define the population at risk, but compromised immunity appears to be a significant risk factor for clinical

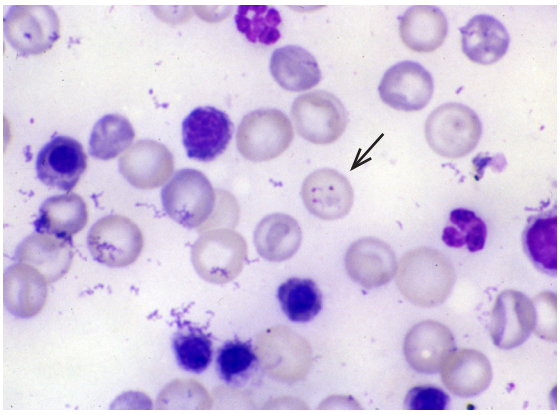

Figure 11.8
Babesia gibsoni (arrow) from a dog. Giemsa. Original magnification x330

disease. The four patients infected by the CA1-type parasite were splenectomized; one died. The transfusion recipients were an elderly man with megaloblastic anemia and myelodysplasia, and a premature infant. The index patient was young and normosplenic. The two blood donors did not have symptoms of babesiosis. Neither reservoir animals nor vector ticks of *B. duncani* have been identified.[28,29,31]

Though reports of babesiosis on the Pacific coast of the US have been rare, it may be that infection by *B. duncani* is more common than by *B. microti*. Seroprevalence studies in asymptomatic persons have revealed prevalent antibody to *B. duncani* in 3.5%, 16%, 17.8% of local populations in northern California.[28,35] In one reference laboratory, 27% of suspected babesiosis cases from across the US have been positive for antibody to *B. duncani*.[36] These findings suggest that infection by *B. duncani*, or a related organism is common.

In 1992 a piroplasm morphologically and serologically similar to *B. divergens* caused a fatal human infection in Missouri. The patient was asplenic and was taking prednisone for long-standing lupus erythematosus. Serologic testing was positive with *B. divergens* and negative with WA1 and *B. microti*. A relatively small (144bp) DNA sequence had 100% similarity to *B. divergens*. The organism could not be propagated in animals that are competent hosts for *B. divergens*. Thus it appeared related to but distinct from *B. divergens*, and was designated MO1.[37] In 2002, *B. divergens* caused a human infection in Kentucky. The patient was asplenic, with possible exposure during a recent hunting trip. Giemsa stained blood smears (Fig 11.4) revealed ring and divergent forms infecting erythrocytes. Its ribosomal RNA gene was 99.8% similar to *B. divergens*.[38]

At least forty cases of human babesiosis have been reported in Europe. Human babesiosis has usually been attributed to cattle *Babesia* (*B. divergens* and *B. bovis* or related piroplasms) transmitted by *Ixodes ricinus*, the castor-bean tick.[39,40] Transovarial transmission has been confirmed, but a particular tick stage has not been implicated (for *Ixodes scapularis*, nymph and seed-tick have been implicated). Residents of areas with large cattle populations appear to have the greatest risk of infection. Asplenic patients had a nearly 50% fatality rate.[40] Serological surveys in Europe have found from 1.5% to 11.5% prevalence of antibodies to *Babesia;*[41-43] serologic findings in Europe suggest exposure to *B. microti*. In the transfusion transmitted case (recipient and donor), *B. microti* was implicated.[17] Given molecular identification of novel parasite species that resemble the large *Babesia* such as *B. divergens*; thus many of the cases historically attributed to *B. divergens* or *B. bovis* were actually caused by a different species.[37,44,45]

Sporadic reports of human babesiosis have come from Canada,[46] Mexico,[23] Cuba,[47] Portugal,[48] Brazil,[49,50] Japan,[51] Czech Republic,[52] Colombia,[53] Italy,[45] Austria,[45] India,[54] Australia,[55] Taiwan,[56,57] China,[40] South Africa,[58] Egypt,[59] the Commonwealth of Independent States,[23] Yugoslavia,[40] Poland,[33] France,[40] Spain,[23,60,61] Germany,[17,62] Korea,[63] Switzerland,[64] Denmark,[65] Sweden,[66] the UK,[40,67] Finland,[68] and Ireland.[39,67,69]

Babesia microti infects children[70,71] and adults at the same frequency, but those with risk factors are more likely to develop clinical, sometimes severe or persistent, disease. Risks include advanced age, asplenism,[21,72] and immunocompromise. Patients with HIV, cancer treatment, immunosuppressive drugs for autoimmune disease, rheumatic disease, or organ transplant have experienced severe babesiosis.[11,21,33,73-81] The incidence of reported *B. microti* infections doubled from more than 200 in 1986 to over 450 by the early 1990s[23,82] and continues to increase in the 2000s.[25] While babesiosis is probably underdiagnosed in all age groups,[83] the increased incidence probably reflects heightened medical and public awareness of the disease and expansion of the endemic range.

Coinfection by *Babesia* and *Borrelia* (Lyme disease agent) have been reported.[84-86] In 1989, a focus of infection emerged in southeastern Connecticut where a large percentage of white-footed mice (*Peromyscus leucopus*) (Fig 11.9) captured near the homes of babesiosis patients were infected with *B. microti.* Some of the mice were also infected with *Borrelia burgdorferi*, the causative agent of Lyme disease.[87] Some of the patients also had serological evidence of recent Lyme disease (IgM to *B. burgdorferi*).

Babesia, *Borrelia*, and *Anaplasma* share a tick vector, and coinfection by two or three of these organisms is known. In 1985, Benach et al reported serological evidence of dual infections in 54% of patients initially diagnosed with babesiosis and 66%, of those initially diagnosed with Lyme disease. They determined that babesiosis and Lyme disease do not cross react and concluded that dual infections were acquired from the same tick.[88] Field surveys have confirmed

that dual infections occur in reservoir animals and vector ticks and there is experimental evidence that vector ticks transmit both infections simultaneously.[89-93] A significant factor in the increasing prevalence of human babesiosis and Lyme disease in the northeastern United States is the growing population of white-tailed deer (*Odocoilus virginianus*) with corresponding increases in the vector tick *lxodes scapularis* (the black-legged or deer tick).[94,95]

Figure 11.9
White-footed mouse (*Peromyscus leucopus*) reservoir host of *Babesia microti* in northern United States.

Infectious Agent

Morphologic Description

With Giemsa or Wright's stains, *Babesia* trophozoites appear as a mass of blue cytoplasm, usually with a single red chromatin dot. Cytoplasmic vacuolation occurs in most species. It is common to find multiple organisms in erythrocytes; 16 or more per red blood cell have been seen in dogs infected with *B. canis*. Unlike plasmodia, neither pigment nor gametocytes are seen in mammalian blood infected with *Babesia*. The genus is generally divided into large (over 3 µm long) and small species (less than 3 µm long), but these categories are not rigid. *Babesia bovis*, a small species infecting cattle, is sometimes as large as *B. bigemina*, a large species also infecting cattle. Large species (and some small ones) are piriform (pear-shaped, "tear-drop" forms), whereas most of the small species are elongate, ameboid, or ring-shaped (annulate). In a single smear, the size range of trophozoites can include both the small and the large sizes. The size and morphology may be influenced by host factors including immune response, splenic presence, and partial treatment.

Babesia microti exhibits considerable pleomorphism (Fig 11.6). Ring (annulate) forms, elongate forms, and multiple chromatin dots are typical features of *B. microti*. The ring forms and ameboid forms of *B. microti* and some of the other small *Babesia* can be morphologically indistinguishable from those of plasmodia (Figs 11.10 & 11.11), and have been misidentified as malaria. Appliqué forms can also be seen, further lending an appearance similar to *Plasmodium falciparum*. The *B. duncani* and (CA1 type parasite) are morphologically similar to *B. microti*. *Babesia duncani* appears in various forms (Fig 11.12 & 11.13), including the Maltese cross (Fig 11.14). Different proportions of the various forms may be observed in infections by *B. microti* and *B. duncani* (described, but data unpublished).[31] In *B. microti* infections, ameboid forms, often with vacuolated cytoplasm, are more common, and tetrad forms are rare and often asymmetrical. The Missouri parasite MO1 is morphologically similar to *B. divergens*.

Babesia that do not produce 4 trophozoites reproduce by binary fission in host erythrocytes. The 2 trophozoites produced in this manner are often seen attached or in close apposition at their narrow ends (Figs 11.2, 11.3, 11.15, & 11.16). Many appear as variously arranged V shapes; *B. divergens* trophozoites are arranged nearly horizontally (Figs 11.4 & 11.17). It is common to find multiple *B. divergens* in a single erythrocyte (Figs 11.4, 11.18, & 11.19). In human erythrocytes, *B. divergens* may appear in ring forms[67] or Maltese cross formations, and are typically located centrally or subcentrally.[96]

Life Cycle and Transmission

The major animal reservoir of human *B. microti* infection in the United States is the white-footed mouse (*Peromyscus leucopus*) (Fig 11.9). Less important are the meadow vole (*Microtus pennsylvanicus*) and other small rodent species.[97,98] If their habitat of brush land, meadows, and pastures is in an endemic area with a white-tailed deer population (host for adult *I. scapularis*), then the wild rodents are likely to be infested with immature stages of *I. scapularis*. This tick, and related species, serves as a transmission vector to host animal (Figs 11.20 & 11.21).

Ticks have a complex life cycle of egg, larva, nymph, and adult stages that may take 2 or more years to complete. With few exceptions, they are 3-host ticks that spend the feeding portion of each stage on a new animal host. Eggs are laid on the ground, where they hatch into tiny larvae (seed ticks) with 3 pairs of legs. These larvae attach firmly to an animal or human, feed once, drop off, and molt into small nymphs with 4 pairs of legs. A year or more later, the nymphs attach to a new host, feed once, drop off, and molt into adults.

There is considerable variation in the feeding and copulation habits of male and female ticks of different genera and species. In general, females require a blood meal to develop eggs. They attach to a host, engorge with blood, drop to the ground, and deposit eggs. On the other hand, many species of adult male *Ixodes* never feed and do not require a blood meal to become fertile. Those that do feed do not engorge,

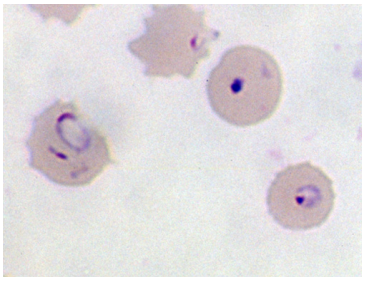

Figure 11.10
Babesia microti in ring form. Note resemblance to early ring form of *P. falciparum* shown in Figure 11.11. Giemsa. Original magnification x330

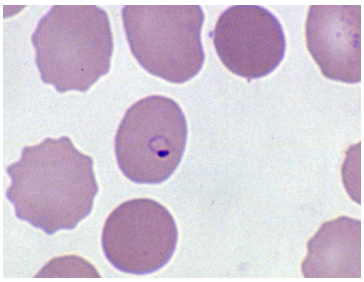

Figure 11.11
Plasmodium falciparum in early ring form. Note resemblance to *Babesia microti* shown in Figure 11.10, and other *Babesia*. Giemsa. Original magnification x330

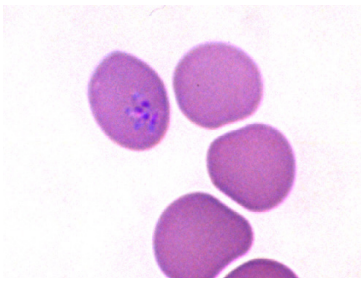

Figure 11.12
Babesia duncani (WA1) causing human babesiosis in Washington State. Morphologically similar to but serologically distinct from, *B. microti* Giemsa. Original magnification x330

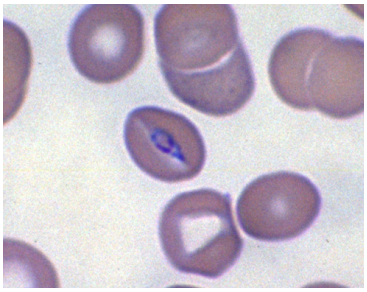

Figure 11.13
Single piriform *Babesia duncani* (WA1) with blue cytoplasm. Giemsa. Original magnification x330

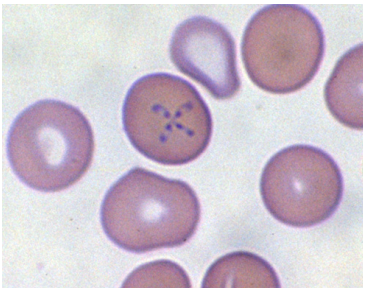

Figure 11.14
Babesia duncani (WA1) in Maltese cross formation. Note dual chromatin dots in each arm of the cross. Giemsa. Original magnification x330

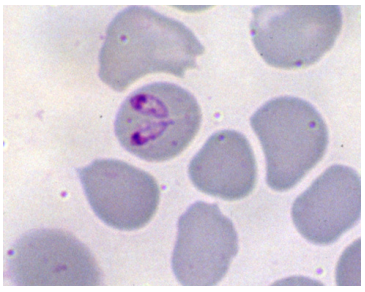

Figure 11.15
Babesia canis from a dog. Giemsa. Original magnification x330

but may take several small meals and copulate with several different females.[99] The mouth parts of male *I. scapularis* are adapted for semen transfer to the female genital opening more than for attachment to a host animal. A male tick attaches to a female throughout her feeding period (a week or more), but leaves her before she drops to the ground. Male ticks remain on a host throughout the winter.[95]

Only larvae that have received a parasite transovarially from an infected female tick can transmit parasites to an animal or human host. Not all *Babesia* are transmitted transovarially; in general, the small species are not. Some tick stages become infected only by feeding on an infected host; they cannot transmit infection until they develop to the next stage and feed again. Once infected, ticks may remain transstadial (infective from stage to stage) through all following stages or only to the next stage. *Babesia microti* are not transmitted transovarially in *I. scapularis*. Larvae are infected by feeding on reservoir animals from July to September and transstadial infection of nymphs follows.

The nymphal stage of *I. scapularis* is the principal vector of *B. microti* to humans.[100,101] In the late 1970s, the vector of *B. microti* in the northeastern United States was thought to be a new tick species called *Ixodes dammini*, but *I. dammini* was later shown to be a variant of *I. scapularis*.[102] Nymphs harbor *B. microti* through the winter and then transmit the organism when feeding during the late spring and early summer.[58,103] Transstadial infection to adult ticks is insignificant and probably only happens when nymphs have fed on an infected animal.[104] Adult *I. scapularis* are strongly host-specific, preferring to feed on their primary host, the white-tailed deer, or some other large animal, thus reducing the likelihood of humans being infected.[95] Most human infections take place in the summer and fall after the most active feeding period of nymphal *I. scapularis*. Nevertheless, a few human infections have appeared in winter months during adult tick feeding.[105]

In the tick, *Babesia* go through many asexual divisions in the gut epithelium, cells of malpighian tubules, hemolymph, and salivary glands. Some *Babesia* develop in the eggs of the female tick and multiply throughout maturing larval tissue, accounting for transovarial transmission. Sexual reproduction of *Babesia* in ticks is still controversial, though sexual stages in the gut epithelium of ticks infected with *B. equi* have been described.[2] After a few days of feeding, tick salivary glands are filled with differentiated sporozoites which are inoculated into the host.

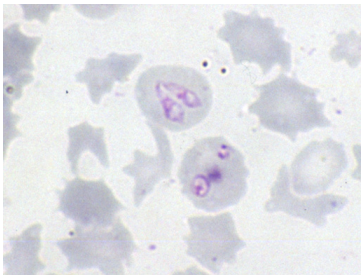

Figure 11.16
Babesia trautmani from pig in Africa. Parasite is significantly larger than small cattle *Babesia*. Giemsa. Original magnification x330

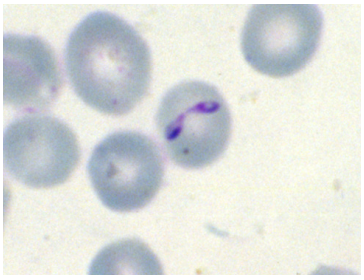

Figure 11.17
Babesia divergens demonstrating nearly linear apposition of 2 trophozoites following binary fission. Giemsa. Original magnification x330

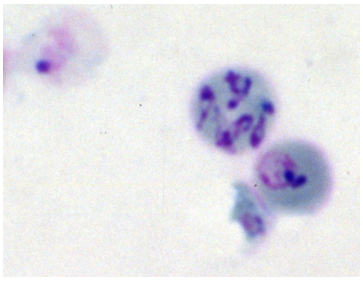

Figure 11.18
Multiple *Babesia divergens* in single erythrocyte. Note piriforms, ring forms, and chromatin dots. Giemsa. Original magnification x330

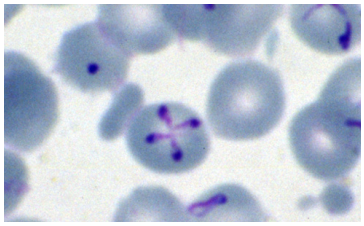

Figure 11.19
Babesia divergens in Maltese cross formation. Giemsa. Original magnification x330

Figure 11.20
Ixodes scapularis, vector of *Babesia microti* to humans. (*Right to left*) millet seed, adult female, adult male, nymph, larva. Millimeter scale.

Figure 11.21 a,b
Same ticks as in Figure 11.20. Note 4 pairs of legs on nymph and adult (a) and 3 pairs of legs on larval tick (b).

Clinical Features and Pathogenesis

Babesiosis provokes a variety of flu-like symptoms: fever, headache, weakness, myalgia, arthralgia, and malaise are common in moderate infections. Severe infections produce anemia, thrombocytopenia, elevated serum hepatic aminotransferase and lactate dehydrogenase, confusion, depression, night sweats, chills, anorexia, nausea, vomiting, splenomegaly (Fig 11.22), and hepatomegaly. The most severe infections may produce respiratory distress, severe icterus, hemoglobinemia, hemoglobinuria (Fig 11.22), oliguria, anuria, prostration, coma, and death.

Humans develop symptoms of babesiosis one to six weeks after being bitten by an infected tick. One to nine weeks is the usual incubation period for transfusion-transmitted infection.[12,106] Infection can range from subclinical and asymptomatic to severe and fatal disease. Mild and asymptomatic infection is detected in seroprevalence studies in communities and volunteer blood donors who report no history of babesiosis, and by transfusion transmission from asymptomatic parasitemic donors. The proportion of infections that are subclinical is unknown; some may be truly asymptomatic, others may have symptoms that were attributed to the "flu" or other illness. Factors limiting the knowledge on incidence and clinical spectrum include variable experience by clinicians and laboratories in recognizing disease and probable species-related virulence.

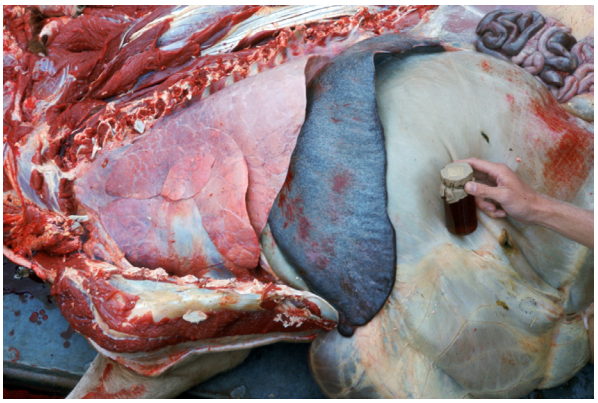

Figure 11.22
Splenomegaly and hemoglobinuria in a cow (urine shown in sample bottle), nearly pathognomonic findings in enzootic areas of babesiosis.

Much of what is known about the pathogenesis of *Babesia* has been a product of veterinary research on *B. bovis*, a highly virulent cattle *Babesia*,[107,108] but some extrapolation can be made to human babesiosis. Most clinical illness is a direct result of hemolysis and interference with erythrocyte function. Parasitic destruction of red blood cell releases pyrogens, causes severe anemia, and may lead to hemoglo-

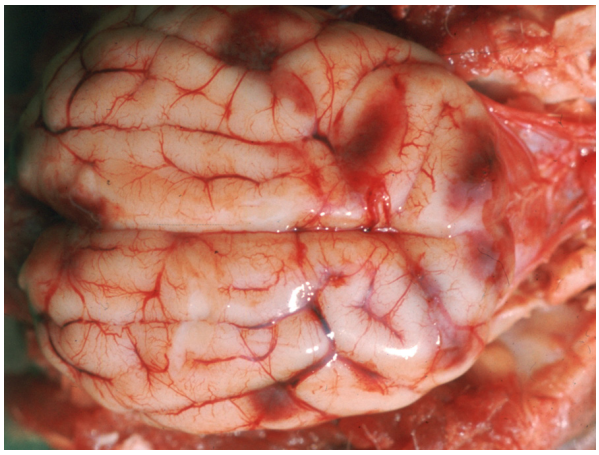

Figure 11.23
Hemorrhages and congestion in leptomeninges of dog infected with *Babesia canis.*

binuric nephrosis. Sludging of parasitized erythrocytes and free parasites in capillaries is one of the most significant events of *Babesia* infections, resulting in serious complications including damaged endothelium, capillary fragility (Fig 11.23), tissue anoxia with degeneration, and an accumulation of toxic byproducts from the host and parasite. A wide variety of factors may precipitate sludging: endothelial fibrin and red blood cell bound fibrinogen; strand-type knob-forming membrane changes in parasitized red blood cells; increased plasma viscosity caused by fibrin, fibrinogen, and their complexes; and erythrocyte lipid changes such as externalization of phosphatidylserine. These factors, plus fibronectin, C3, conglutin, parasite antigen, IgG, and cryofibrinogen, have been found in cattle with cerebral babesiosis caused by *B. bovis*.[107,108] The cellular immune system, including production of cytotoxic TNF-alpha, plays an important role in many of these clinical changes. Large amounts of antibody are produced in cattle. *Babesia bovis* produces a more virulent disease in nonsplenectomized adult cattle than in weaned, splenectomized calves, supporting the argument that the most serious symptoms of *B. bovis* infection are related to the immune response.[108]

Cytokine studies in a human patient in the acute stage of babesiosis revealed elevated levels of TNF-alpha, interferon-gamma, IL2, IL6, E selectin, vascular adhesion moIecule-1, and intercellular adhesion molecule-1. CD8+ T cells and natural killer cells were also present.[73,103] A small but growing number of human babesiosis patients have severe respiratory complications.[109,110] Parasite and host-derived proteases, plasma kallikrein, thrombin and thrombin-like enzymes, and anoxia cause alveolar edema and an accumulation of neutrophils and red blood cells in pulmonary capillaries.[108] Increased resistance of erythrocytes to deformation, increased cytoadherence, and perhaps excessive production of TNF and IL-1, may participate in the pathogenesis of respiratory distress in human patients.[109]

Other complications of babesiosis have included retinal infarct, encephalopathy, hepatic failure, renal failure, hemophagocytic syndrome,[111] and autoimmune hemolytic anemia.[112]

The development and severity of human babesiosis is influenced by multiple factors, including virulence of the species of *Babesia*, a compromised immune system, asplenia, corticosteroid or other immunosuppressive therapy, HIV infection, and coinfection with other pathogen.[33,113,114] *Babesia microti* is more likely than other species to produce clinical disease in the elderly. Young adult and middle-aged males (the group with the highest tick exposure) are the usual victims of the more virulent species (*B. bovis* and *B. divergens*). Within this group, asplenic males are at greatest risk of severe or fatal disease. Coinfection with babesiosis and Lyme borreliosis occurs commonly, but triple infection is uncommon. Some reports suggest that the course of Lyme disease is more severe or prolonged with *Babesia* coinfection, others disagree.[86,115,116]

The reticuloendothelial system plays a vital role in nonspecific resistance. Specific resistance depends on both humoral and cell-mediated immunity. *Babesia* infection elevates levels of IgG and IgM in animals and humans. In vitro observations suggest that immunity may be influenced by humoral factors; phagocytosis of infected cells by splenic macrophages requires antibody, complement, and conglutin.[108] In mice, T-cell lymphocytes are responsible for primary resistance to *B. microti*.[117]

Pathologic Features

In humans, findings at autopsy include congestion of deep organs, occasional hemorrhage, hyperplastic lymph nodes, severe icterus of the skin, mucous membranes, and serosal and visceral surfaces, severe pulmonary edema, enlarged yellow liver, swollen kidneys with reddish brown discoloration of the corticomedullary junction (Fig 11.24), dark urine in the bladder, swelling of the brain, and icteric meninges. Microscopic findings include parasitized erythrocytes in congested capillaries of multiple organs (especially liver sinusoids), acute renal tubular necrosis, hemoglobin casts in tubules, distention of liver canaliculi by bile, immature blood cells in vessels, disseminated intravascular coagulation, and hemophagocytosis.[39,103,111,118] *Babesia* are usually apparent in hematoxylin-eosin stained sections, but are best seen with the Giemsa stain.

Diagnosis

Babesiosis must be considered in a patient with malaria-like symptoms and a history of travel to regions endemic

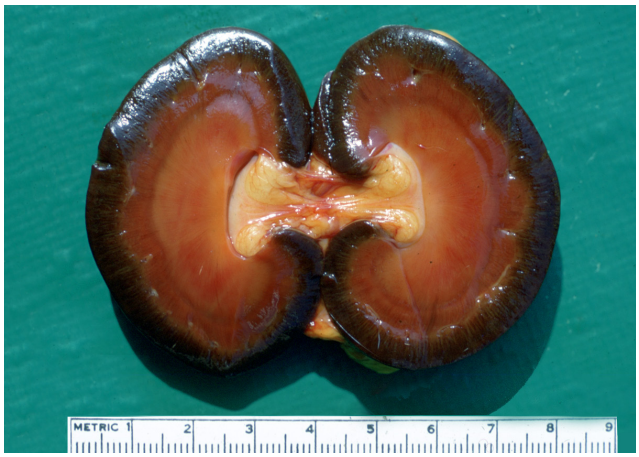

Figure 11.24
Cut surface of kidney from a dog. Note dark red hemoglobin-stained cortical surface and reddish brown discoloration of corticomedullary junction.

for babesiosis or recent transfusion. Because of the similarity of symptoms and geographic distribution of babesiosis, Lyme disease, and anaplasmosis; suspicion of one disease should prompt testing for all.

The laboratory tests that can be used to diagnose infection by *Babesia* include light microscopy of peripheral blood, serologic identification of anti-*Babesia* antibodies, and detection of *Babesia* nucleic acid. No test kit or device for diagnosis of *Babesia* infection is commercially available. The FDA has not approved any method. Some research and commercial reference laboratories have "home grown" testing capability. The most appropriate test in most settings is light microscopy and it is still the "Gold Standard". WHO and CDC recommend the following:

Microscopy.[119] For microscopic examination, peripheral blood is spread on slides in thick and thin smears. At least two of each should be made. Blood smears are useful for detection and identification of a number of different blood parasites. The preferred stain is Giemsa, which can reveal Schüffner's dots of malaria. Other stains (Wright, Wright-Giemsa, Field's stain or other Romanowsky stains) can also be used. The guidelines recommend examining the slide with an oil 100x objective (1000x magnification). Small parasites of either *Babesia* or *Plasmodium* can be easily missed at lower magnification. Examination of a thick smear is approximately 20 times more sensitive than a thin smear. WHO recommends examining at least 100 fields of a thick smear before calling a smear negative.[120] Clinical and Laboratory Standards Institute (formerly NCCLS) standards recommend examining at least 300 fields of a thin smear and a thick smear.[121] The estimated volume of a thick smear examined at 1000x for 100 fields is 0.28 µl.[122] If one parasite is seen, the estimated mean limit of detection is about 4 parasites/µl. The thin smear is often required to provide better visualization of parasite morphology for identification. Malaria and babesia ring forms can be difficult or impossible to distinguish one from the other.

Serology

Detection of antibody has traditionally been done by immununofluorescent antibody (IFA) test. The recommended cutoff titres differ by test. However, there is strong cross-reactivity of *B. microti* and several *Plasmodium* sp,[123] so IFA testing in areas endemic for both malaria and babesiosis is inconclusive. More specific tests may be useful in differentiating babesiosis from malaria when microscopic examination and travel history do not support or exclude either parasite. An IFA test has been developed for *B. duncani* (WA1) that has no cross-reactivity with *B. microti* antigen. Serum from patients seropositive to WA1 is cross-reactive with *B. gibsoni* antigen but at significantly lower titers.[28]

The substrate for the IFA has traditionally been obtained by inoculation of susceptible animals (Syrian hamster) and using the infected red cells when high (e.g. 40%) parasitemia is achieved. This can take several weeks. The red cells are washed and concentrated and spread on a slide as a thick smear. Alternatively, relatively purified parasites from the red cells can be used. Recombinant peptides representing specific epitopes have also been tested.[124] Antibody detection using an enzyme-linked detection test (ELISA, EIA) has been performed and may be adaptable to existing platforms used in routine clinical laboratories.

Direct detection of *Babesia* antigens is not available; however, an antigen detection kit for *Plasmodium* (e.g. BinaxNow® rapid diagnostic test) can be useful, since babesia and plasmodia can be morphologically similar. The presence of *Plasmodium* antigen can help differentiate *Babesia* and malaria.[125]

Fluorescent dye detection.

Dyes that stain nucleic acids can be used to detect blood parasites. The QBC® test kit described in Topic 10 also detects *Babesia*. It is a nonspecific screening test. Routine light microscopy follows the QBC® test to confirm and identify the parasite.

Molecular detection.

An 18S RNA gene sequence has been helpful in identifying species of *Babesia* and related protozoa. Protocols for polymerase chain reactions (PCR) have been used in research and by commercial reference laboratories; however, no commercially available test kits are available. *Babesia*-specific DNA probes have been a significant investigative tool in the study of novel species (e.g. WA1, CA1, MO1, EU1) and phylogenetics.[28-30] DNA probes and PCR tests can be useful in diagnosing low parasitemic infections.[126,127]

Molecular diagnosis is not a primary diagnostic test. Its use should be reserved for the following situations:

- Cases in which organisms are not identified by microscopy and there is a strong clinical suspicion (presumed low parasitemia).[119]
- When *Babesia* and malaria cannot be differentiated by morphology or clinical history, e.g. patient has no known risk for either or has risk for both.[119]
- In recurrent disease after treatment or failure after treatment, to determine if the original diagnosis was incorrect.
- In investigational research of new *Babesia* species or related parasites.[119]
- To reduce risk of transfusion transmission by testing blood donors.[16,128]

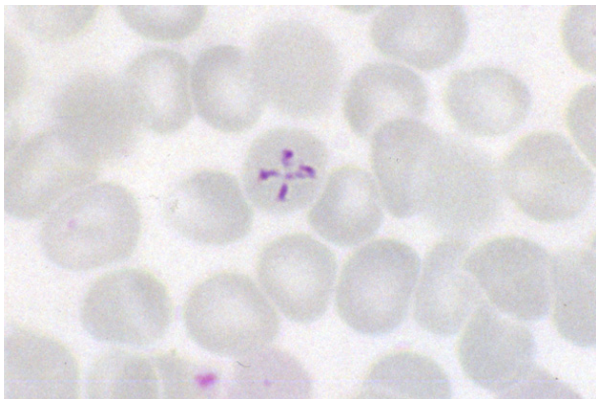

Figure 11.25
Babesia rodhaini in Maltese cross formation. Formation may be difficult to see in thin thin blood film. Giemsa. Original magnification x330

Identifying species on the basis of morphology is difficult because of similarities among *Babesia* species. In the United States, *B. microti* infection can be diagnosed with certainty if it occurs in a recognized *B. microti* endemic region and the parasite displays a characteristic tetrad formation (Fig 11.5). Finding the Maltese cross formation is helpful but requires meticulous searching (Fig 11.25)

Treatment and Prevention

The current treatment of choice for most symptomatic human *Babesia* infections is atovaquone plus azityhromycin. Because of a higher rate of adverse drug effects, the combination of clindamycin plus quinine should be reserved for failure by the first line treatment. For severe babesiosis, clindamycin should be given intravenously. Red cell exchange may be considered to reduce parasite burden.[129]

The adult dosage of atovaquone is 750 mg orally every 12 hours; azithromycin is given at 500 to 1000 mg first dose followed by 250 mg daily. Clindamycin is given 300-600 mg every 6 hours (IV) or 600 mg every 6-8 hours orally; quinine, 650 mg, is given every 6-8 hours orally. Higher doses of azithromycin (600 to 1000 mg daily) may be used for immunocompromised patients.[129]

In conjunction with exchange transfusions, this combination of drugs has successfully treated human patients with severe *B. divergens* and *B. microti* infections.[40,75,130] Azithromycin plus quinine cleared *Babesia* parasites from the blood of a Taiwanese patient.[131] Azithromycin and atovaquone, used in combination with or without quinine, have effectively treated babesiosis in adult, infant, asplenic, and HIV-positive patients where clindamycin plus quinine had failed.[22,131-133] Trimethoprim and sulfamethoxazole successfully treated a transplant patient with a *B. microti* infection after other drug therapies had failed.[118] Pentamidine alone has not been successful,[134] but pentamidine plus cotrimoxazole (trimethoprim) and sulfamethoxazole effectively treated a splenectomized patient infected with *B. divergens*. Immunocompetent patients may clear parasitemia without any treatment. In some instances, exchange transfusion is needed to rid patients of persistent parasitemia.[9,74]

There is no vaccine for human babesiosis. The best prevention is to promote public awareness in endemic regions and areas where *Ixodes* ticks (or other competent vectors) are abundant. Simple precautions include using insect repellents, especially in the spring and summer months, checking for ticks after exposure to their habitat, and wearing light-colored clothing to make crawling ticks more visible. Control measures include protecting human habitats by eliminating nesting areas for small reservoir rodents and excluding deer with adequate fencing. Reducing the tick population with acaricides is recommended, especially around lawns and residences.

Blood collectors and regulatory agencies in the US are exploring options for reducing the risk of transfusion transmission of *Babesia*. Proposed strategies include various testing protocols to exclude donations from potentially infected blood donors and modification of the collected blood to destroy pathogens or prevent their replication (pathogen reduction).[16,128]

References

1. Mehlhorn H, Schein E. Redescription of *Babesia equi Laveran*, 1901 as *Theileria equi Mehlhorn*, Schein 1998. *Parasitol Res*. 1998;84:467-475.
2. Mehlhorn H, Schein E. The piroplasms: life cycle and sexual stages. *Adv Parasitol*. 1984;23:37-103.
3. Skrabalo Z. Babesiosis. In: Marcial-Rojas RA, Moreno E, eds. *Pathology of Protozoal and Helminthic Diseases with Clinical Correlation*. Baltimore, Md: Williams & Wilkins; 1971:225-233.
4. Smith T, Kilbourne FL. Investigations into the nature, causation and prevention of Texas or southern cattle fever. *US Dept Agr Bur Anim Indust*. 1893;Bull 1:1-301.
5. Wilson LB, Chowning WM. Studies in pyroplasmosis hominis (spotted fever or tick fever of the Rocky Mountains). *J Infect Dis*. 1904;1:31-57.
6. Scholtens RG, Braff EH, Healey GA, Gleason N. A case of babesiosis in man in the United States. *Am J Trop Med Hyg*. 1968;17:810-813.
7. Fitzpatrick JE, Kennedy CC, McGeown MG, Oreopoulos DG, Robertson JH, Soyannwo MA. Human case of piroplasmosis (babesiosis). *Nature*. 1968;217:861-862.
8. Western KA, Benson GD, Gleason NN, Healy GR, Schultz MG. Babesiosis in a Massachusetts resident. *N Engl J Med*. 1970;283:854-856.
9. Jacoby GA, Hunt JV, Kosinski KS, et al. Treatment of transfusion-transmitted babesiosis by exchange transfusion. *N Engl J Med.* 1980;303:1098-1100.
10. Dobroszycki J, Herwaldt BL, Boctor F, et al. A cluster of transfusion-associated babesiosis cases traced to a single asymptomatic donor. *JAMA*. 1999;281:927-930.
11. Herwaldt BL, Kjemtrup AM, Conrad PA, et al. Transfusion-transmitted babesiosis in Washington State: first reported case caused by a WA1-type parasite. *J Infect Dis*. 1997;175:1259-1262.
12. Mintz ED, Anderson JF, Cable RG, Hadler JL. Transfusion-transmitted babesiosis: a case report from a new endemic area. *Transfusion*. 1991;31:365-368.
13. Asad S, Sweeney J, Mermel LA. Transfusion-transmitted babesiosis in Rhode Island. *Transfusion*. 2009;49:2564-2573.
14. Tonnetti L, Eder AF, Dy B, et al. Transfusion-transmitted *Babesia microti* identified through hemovigilance. *Transfusion*. 2009;49:2557-2563.
15. Young C and Krause PJ. Editorial: The problem of transfusion-transmitted babesiosis. *Transfusion*. 2009;49:2548–2550.
16. American Association of Blood Banks. Summary of Blood Products Advisory Committee Meeting 7/26 - 7/27/10. http://www.aabb.org/events/government/bpac/Pages/bpacmeeting072610.aspx. Accessed March 7, 2011
17. Hildebrandt A, Hunfeld KP, Baier M, et al. First confirmed autochthonous case of human *Babesia microti* infection in Europe. *Eur J Clin Microbiol Infect Dis.* 2007;26:595-601.
18. Saito-Ito A, Tsuji M, Wei Q, et al. Transfusion-acquired, autochthonous human babesiosis in Japan: isolation of *Babesia microti*-like parasites with hu-RBC-SCID mice. *J Clin Microbiol*. 2000;38:4511-4516.
19. DeVos AJ, Imes GD Jr, Culien JS. Cerebral babesiosis in a newborn calf. *Onderstepoort J Vet Res.* 1976;43:75-78.
20. U.S. Department of Health and Human Services Center for Disease Control. Babesiosis becomes notifiable. http://www.cdc.gov/parasites/features/babesia_notify_9-7-10.html. Page last reviewed: November 2, 2010. Page last updated: November 2, 2010. Accessed March 7, 2011.
21. Meldrum SC, Birkhead GS, White DJ, Benach JL, Morse DL. Human babesiosis in New York State: an epidemiological description of 136 cases. *Clin Infect Dis.* 1992;15:1019-1023.
22. New DL, Quinn JB, Qureshi MZ, Sigler SJ. Vertically transmitted babesiosis. *J Pediatr.* 1997;131:163-164.
23. Healy GR, Ristic M. Human babesiosis. In: Ristic M, ed. *Babesiosis of Domestic Animals and Man*. Boca Raton, Fla: CRC Press, Inc; 1988:209-225.
24. Herwaldt BL, McGovern PC, Gerwel MP, Easton RM, MacGregor RR. Endemic babesiosis in another eastern state: New Jersey. *Emerg Infect Dis.* 2003;9:184-188.
25. Rodgers SE, Mather TN. Human *Babesia microti* incidence and *Ixodes scapularis* distribution, Rhode Island, 1998–2004. *Emerg Infect Dis.* 2007;13:633–635.
26. Weld ED, Eimer KM, Saharia K, Orenstein A, Hess JR. The expanding range and severity of babesiosis. *Transfusion*. 2010;50:290–291.
27. Bredt AS, Weinstein WM, Cohen S. Treatment of babesiosis in asplenic patients. *JAMA*. 1981;254:1938-1939.
28. Persing DH, Herwaldt BL, Glaser C, et al. Infection with a babesia-like organism in northern California. *N Engl J Med*. 1995;332:298-303.
29. Quick RE, Herwaldt BL, Thomford JW, et al. Babesiosis in Washington State: a new species of Babesia? *Ann Intern Med.* 1993;119:284-290.
30. Thomford JW, Conrad PA, Telford SR 3d, et al. Cultivation and phylogenetic characterization of a newly recognized human pathogenic protozoan. *J Infect Dis.* 1994;169:1050-1056.
31. Conrad PA, Kjemtrup AM, Carreno RA, et al. Description of *Babesia duncani* n.sp. (Apicomplexa: Babesiidae) from humans and its differentiation from other piroplasms. *Int J Parasitol*. 2006;36:779-789.
32. Jerant AF, Arline AD. Babesiosis in California. *West J Med*. 1993;158:622-625.
33. Homer MJ, Aguilar-Delfin I, Telford SR 3d, Krause PJ, Persing DH. Babesiosis. *Clin Microbiol Rev.* 2000;13:451-469.
34. Kjemtrup AM, Lee B, Fritz CL, Evans C, Chervenak M, Conrad PA. Investigation of transfusion transmission of a WA1-type babesial parasite to a premature infant in California. *Transfusion*. 2002;42:1482-1487.
35. Fritz CL, Kjemtrup AM, Conrad PA, et al. Seroepidemiology of emerging tickborne infectious diseases in a Northern California community. *J Infect Dis.* 1997;175:1432-1439.
36. Prince HE, Lapé-Nixon M, Patel H, Yeh C. Comparison of the *Babesia duncani* (WA1) IgG detection rates among clinical sera submitted to a reference laboratory for WA1 IgG testing and blood donor specimens from diverse geographic areas of the United States. *Clin Vaccine Immunol*. 2010;17:1729-1733.
37. Herwaldt B, Persing DH, Precigout EA, et al. A fatal case of babesiosis in Missouri: identification of another piroplasm that infects humans. *Ann Intern Med.* 1996;124:643-650.
38. Beattie JF, Michelson ML, Holmes PJ. Acute babesiosis caused by *Babesia divergens* in a resident of Kentucky. *N Engl J Med.* 2002;347:697-698.
39. Clarke CS, Rogers ET. Egan EL. Babesiosis: under-reporting or case-clustering? *Postgrad Med J.* 1989;65:591-593.
40. Gorenflot A, Moubri K, Precigout E, Carcy B, Schetters TP. Human babesiosis. *Ann Trop Med Parasitol*. 1998;92:489-501.
41. Hunfeld KP, Lambert A, Kampen H, et al. Seroprevalence of Babesia infections in humans exposed to ticks in midwestern Germany. *J Clin Microbiol.* 2002;40:2431-2436.
42. Foppa IM, Krause PJ, Spielman A, et al. Entomologic and serologic evidence of zoonotic transmission of *Babesia microti*, eastern Switzerland. *Emerg Infect Dis.* 2002;8:722-726.
43. Genchi C. Human babesiosis, an emerging zoonosis. *Parassitologia*. 2007;49(Suppl 1):29-31.
44. Herwaldt BL. Epidemiology of Babesiosis, Presentation to Blood Products Advisory Committee in July 2010. Document ucm222400. Available at http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/BloodProductsAdvisoryCommittee/ucm205013.htm. Last Updated: 01/10/2011. Accessed March 17, 2011.
45. Piccaluga PP, Poletti G, Martinelli G, Gherlinzoni F. Babesia infection in Italy. *Lancet Infect Dis*. 2004;4:212
46. Bu Jassoun S, Fong IW, Hannach B, Kain KC. Transfusion-transmitted babesiosis in Ontario: first reported case in Canada. *Can Commun Dis Rep*. 2000;26:9-13.
47. Suarez Hernandez M, Alonso Castellano M, Pelaez Martinez R, Sanchez Perez B, Bravo Gonzalez JR, Sanchez Sibello A. Investigation of Babesia in farm workers and blood donors in the province of Ciego de Avila [in Spanish]. *Rev Cubana Med Trop*. 1997;49:130-135.
48. Centeno-Lima S, do Rosário V, Parreira R,et al. A fatal case of human babesiosis in Portugal: molecular and phylogenetic analysis. *Trop Med Int Health*. 2003;8:760-764.
49. Yoshinari NH, Abrão MG, Bonoldi VL, Soares CO, et al. Coexistence of antibodies to tick-borne agents of babesiosis and Lyme borreliosis in patients from Cotia county, State of São Paulo, Brazil. *Mem Inst Oswaldo Cruz*. 2003;98:311-318.
50. Rech A, Bittar CM, de Castro CG, et al. Asymptomatic babesiosis in a child with hepatoblastoma. *J Pediatr Hematol Oncol*. 2004;26:213.
51. Matsui T, Inoue R, Kajimoto K, et al. [First documentation of transfusion-associated babesiosis in Japan] [Article in Japanese, abstract in English]. *Rinsho Ketsueki*. 2000;41:628-634.
52. Nohýnková E, Kubek J, Měst'ánková O, Chalupa P, Hubálek Z. [A case of *Babesia microti* imported into the Czech Republic from the USA] [Article in Czech, Abstract in English]. *Cas Lek Cesk*. 2003;142:377-381.

53. Ríos L, Alvarez G, Blair S. Serological and parasitological study and report of the first case of human babesiosis in Colombia. *Rev Soc Bras Med Trop*. 2003;36:493-498.

54. Marathe A, Tripathi J, Handa V, Date V. Human babesiosis: a case report. *Indian J Med Microbiol*. 2005;23:267-269.

55. Clark IA, Budd AC, Hsue G, et al. Absence of erythrocyte sequestration in a case of babesiosis in a splenectomized human patient. *Malar J*. 2006;5:69.

56. Shaio MF, Lin PR. A case study of cytokine profiles in acute human babesiosis. *Am J Trop Med Hyg*. 1998;58:335-337.

57. Shih CM, Liu LP, Chung WC, Ong SJ, Wang CC. Human babesiosis in Taiwan: asymptomatic infection with a *Babesia microti*-like organism in a Taiwanese woman. *J Clin Microbiol*. 1997;35:450-454.

58. Bush JB, Isaacson M, Mohamed AS, Potgieter FT, de Waal DT. Human babesiosis -a preliminary report of 2 suspected cases in South Africa. *S Afr Med J*. 1990;78:699.

59. El-Bahnasawy MM, Morsy TA. Egyptian human babesiosis and general review. *J Egypt Soc Parasitol*. 2008;38:265-272.

60. Canas E, de Alarcon A, Regordan C. Viciana P. Babesiosis in an immunocompetent patient [in Spanish] [letter]. *Rev Clin Esp.* 1992;190:45.

61. Moreno Giménez JC, Jiménez Puya R, Galán Gutiérrez M, Ortega Salas R, Dueñas Jurado JM. *Erythema figuratum* in septic babesiosis. *J Eur Acad Dermatol Venereol*. 2006;20:726-728.

62. Häselbarth K, Tenter AM, Brade V, Krieger G, Hunfeld KP. First case of human babesiosis in Germany: clinical presentation and molecular characterization of the pathogen. *Int J Med Microbiol*. 2007;297:197-204.

63. Kim JY, Cho SH, Joo HN, et al. First case of human babesiosis in Korea: detection and characterization of a novel type of *Babesia* sp (KO1) similar to ovine babesia. *J Clin Microbiol*. 2007;45:2084-2087.

64. Loutan L, Rossier J, Zufferey G, et al. Human babesiosis: first case report in Switzerland [in French]. Rev Med Suisse Romande. 1994;114:111-116.

65. Enemark H, Petersen JE. Babesiosis in Denmark [in Danish]. *Ugeskr Laeger.* 1998;160:6225.

66. Uhnoo I, Cars O, Christensson D, Nystrom-Rosander C. First documented case of human babesiosis in Sweden. *Scand J Infect Dis.* 1992;24:541-547.

67. Loutan L, Rossier J, Zufferey G, et al. Imported babesiosis diagnosed as malaria [letter]. *Lancet*. 1993;342:749.

68. Haapasalo K, Suomalainen P, Sukura A, Siikamaki H, Jokiranta TS. Fatal babesiosis in man, Finland, 2004. *Emerg Infect Dis*. 2010;16:1116-1118.

69. Browne S, Ryan Y, Goodyer M, Gilligan O. Fatal babesiosis in an asplenic patient. *Br J Haematol.* 2010;148:494.

70. Esernio-Jenssen D, Scimeca PG, Benach JL, Tenenbaum MJ. Transplacental/ perinatal babesiosis. *J Pediatr.* 1987;110:570-572.

71. Scimeca PG, Weinblatt ME, Schonfeld G, Kaplan MH, Kochen JH. Babesiosis in two infants from eastern Long Island, NY [letter]. *Am J Dis Child*. 1986;140:971.

72. Benach JL, Habicht GS. Hamburger MI. Immunoresponsiveness in acute babesiosis in humans. *J Infect Dis.* 1982;146:369·380.

73. Benezra D, Brown AE, Polsky B, Gold JW, Armstrong D. Babesiosis and infection with human immunodeficiency virus [letter]. *Ann Intern Med.* 1987;107:944.

74. Machtinger L, Telford SR 3d, Inducil C, Klapper E, Pepkowitz SH, Goldfinger D. Treatment of babesiosis by red blood cell exchange in an HlV-positive, splenectomized patient. *J Clin Apheresis.* 1993;8:78-81.

75. Ong KR, Stavropoulos C, Inada Y. Babesiosis, asplenia, and AIDS [letter]. *Lancet*. 1990;336:112.

76. Lux JZ, Weiss D, Linden JV, et al. Transfusion-associated babesiosis after heart transplant. *Emerg Infect Dis*. 2003;9:116-119.

77. Taiwo B, Lee C, Venkat D, Tambar S, Sutton SH. Can tumor necrosis factor alpha blockade predispose to severe babesiosis? *Arthritis Rheum*. 2007;57:179-181.

78. Häselbarth K, Kurz M, Hunfeld KP, Krieger G. Babesiosis in an immunocompromised German patient (Article in German, Abstract in English). *Med Klin (Munich)*. 2008;103:104-107.

79. Cullen G, Sands BE, Yajnik V. Babesiosis in a patient on infliximab for Crohn's disease. *Inflamm Bowel Dis*. 2010;16:1269-1270.

80. Berman KH, Blue DE, Smith DS, Kwo PY, Liangpunsakul S. Fatal case of babesiosis in post liver transplant patient. *Transplantation*. 2009;87:452-453.

81. Perdrizet GA, Olson NH, Krause PJ, Banever GT, Spielman A, Cable RG. Babesiosis in a renal transplant recipient acquired through blood transfusion. *Transplantation*. 2000;70:205-208.

82. Spach DH, Liles WC, Campbell GL, Quick RE, Anderson DE Jr, Fritsche TR. Tick-borne diseases in the United States [review]. *N Engl J Med*. 1993;329:936-947.

83. Krause PJ, Telford SR 3d, Pollack RJ, et al. Babesiosis: an underdiagnosed disease of children. *Pediatr*. 1992;89:1045-1048.

84. Grunwaldt E, Barbour AG, Benach JL. Simultaneous occurrence of babesiosis and Lyme disease [letter]. *N Engl J Med.* 1983;308:1166.

85. Marcus LC, Steere AC, Duray PH, Anderson AE, Mahoney EB. Fatal pancarditis in a patient with coexistent Lyme disease and babesiosis. Demonstration of spirochetes in the myocardium. *Ann Intern Med*. 1985;103:374-376.

86. Krause PJ, Telford SR 3rd, Spielman A, et al. Concurrent Lyme disease and babesiosis. Evidence for increased severity and duration of illness. *JAMA*. 1996;275:1657-1660.

87. Anderson JF, Mintz ED, Gadbaw JJ, Magnarelli LA. *Babesia microti*, human babesiosis, and *Borrelia burgdorferi* in Connecticut. *J Clin Microbiol*. 1991;29:2179-2783

88. Benach JL, Coleman JL, Habitat GS, MacDonald A, Grunwaldt E, Giron JA. Serological evidence for simultaneous occurrences of Lyme disease and babesiosis. *J Infect Dis*. 1985;152:473-477.

89. Anderson JF, Johnson RC, Magnarelli LA, Hyde FW, Myers JE. Prevalence of *Borrelia burgdorferi* and *Babesia microti* in mice on islands inhabited by white-tailed deer. *Appl Environ Microbiol.* 1987;53:892-894.

90. Mather TN, Telford SR 3d, Moore Sl, Spielman A. *Borrelia burgdorferi* and *Babesia microti*: efficiency of transmission from reservoirs to vector ticks (*Ixodes dammini*). *Exp Parasitol.* 1990;70:55-61.

91. Piesman J. Intensity and duration of *Borrelia burgdorferi* and *Babesia microti* infectivity in rodent hosts. *Int J Parasitol*. 1988;18:687-689.

92. Piesman J, Hicks TC, Sinsky RJ, Obiri G. Simultaneous transmission of *Borrelia burgdorferi* and *Babesia microti* by individual nymphal *Ixodes dammini* ticks. *J Clin Microbiol*. 1987;25:2012-2013.

93. Piesman J, Mather TN, Telford SR 3d, Spielman A. Concurrent *Borrelia burgdorferi* and *Babesia microti* infection in nymphal *Ixodes dammini. J Clin Microbiol.* 1986;24:446-447.

94. Piesman J, Spielman A, Etkind P, Ruebush TK 2d, Juranek DD. Role of deer in the epizootiology of *Babesia microti* in Massachusetts, USA. *J Med Entomol.* 1979;15:537-540.

95. Spielman A, Wilson ML, Levine JF, Piesman J. Ecology of *Ixodes dammini*-borne human babesiosis and Lyme disease. *Annu Rev Entomol*. 1985;30:439-460.

96. Brasseur P, Gorenflot A. Human babesiosis in Europe. *Mem Inst Oswaldo Cruz*. 1992;87:131-132.

97. Spielman A, Etkind P, Piesman J, Ruebush TK 2d, Juranek DD, Jacobs MS. Reservoir hosts of human babesiosis on Nantucket Island. *Am J Trop Med Hyg*. 1981;30:560-565.

98. Telford SR 3d, Mather TN, Adler GH, Spielman A. Short-tailed shrews as reservoirs of the agents of Lyme disease and human babesiosis. *J Parasitol*. 1990;76:681-683.

99. Oliver JH Jr. Symposium on reproduction of arthropods of medical and veterinary importance. IV. Reproduction in ticks (Ixodoidea). *J Med Entomol*. 1974;11:26-34.

100. Piesman J, Spielman A. Human babesiosis on Nantucket Island: prevalence of *Babesia microti* in ticks. *Am J Trop Med Hyg.* 1980;29:742-746.

101. Spielman A. Human babesiosis on Nantucket Island: transmission by nymphal Ixodes ticks. *Am J Trop Med Hyg*. 1976;25:784-787.

102. Oliver JH Jr, Owsley MR, Hutcheson HJ, et al. Conspecificity of the ticks *Ixodes scapularis* and *I. dammini* (Acari: Ixodidae). *J Med Entomol*. 1993;30:54-63.

103. Auerbach M, Haubenstock A, Soloman G. Systemic babesiosis. Another cause of the hemophagocytic syndrome. *Am J Med*. 1986;80:301-303.

104. Piesman J, Karakashian SJ, Lewengrub S, Rudzinska MA, Spielman A. Development of *Babesia microti* sporozoites in adult *Ixodes dammini*. *Int J Parasitol*. 1986;16:381-385.

105. Piesman J, Mather TN, Dammin GJ, Telford SR 3d, Lastavica CC, Spielman A. Seasonal variation of transmission risk of Lyme disease and human babesiosis. *Am J Epidemiol*. 1987;126:1187-1189.

106. Leiby DA. Babesiosis and blood transfusion: flying under the radar. *Vox Sang.* 2006;90:157-165.

107. Wright IG, Goodger BV. Pathogenesis of babesiosis. In: Ristic M, ed. *Babesiosis of Domestic Animals and Man.* Boca Raton, Fla: CRC Press, Inc; 1988:99-118.

108. Wright IG, Goodger BV, Buffington GD, Clark IA, Parrodi F, Waltisbuhl DJ. Immunopathophysiology of babesial infections. *Trans R Soc Trop Med Hyg.* 1989;83:11-13.

109. Boustani MR, Lepore TJ, Gelfand JA, Lazarus DS. Acute respiratory failure in patients treated for babesiosis. *Am J Respir Crit Care Med*. 1994;149:1689-1691.

110. Horowitz ML, Coletta F, Fein AM. Delayed onset adult respiratory distress syndrome in babesiosis. *Chest.* 1994;106:1299-1301.

111. Slovut DP, Benedetti E, Matas AJ. Babesiosis and hemophagocytic syndrome in an asplenic renal transplant recipient. *Transplantation.* 1996;62:537-539.

112. Evenson DA, Perry E, Kloster B, Hurley R, Stroncek DF. Therapeutic apheresis for babesiosis. *J Clin Apher*. 1998;13:32-36.

113. Babesiosis-Connecticut. *MMWR Morb Mortal Wkly Rep*. 1989;38:649-650.

114. Rosner F, Zarrabi MH, Benach JL, Habicht GS. Babesiosis in splenectomized adults. Review of 22 reported cases. *Am J Med*. 1984;76:696-701.

115. Swanson SJ, Neitzel D, Reed KD, Belongia EA. Coinfections acquired from Ixodes ticks. *Clin Microbiol Rev*. 2006;19:708–727.

116. Wang TJ, Liang MH, Sangha O, et al. Coexposure to *Borrelia burgdorferi* and *Babesia microti* does not worsen the long-term outcome of lyme disease. *Clin Infect Dis.* 2000;31:1149-1154.

117. James MA. Immunology of babesiosis. In: Ristic M, ed. *Babesiosis of Domestic Animals and Man*. Boca Raton, Fla: CRC Press, Inc; 1988:119-130.

118. Gupta P, Hurley RW, Helseth PH, Goodman JL, Hammerschmidt DE. Pancytopenia due to hemophagocytic syndrome as the presenting manifestation of babesiosis. *Am J Hematol*. 1995;50:60-62.

119. Centers for Disease Control and Prevention. Laboratory Identification of Parasites of Public Health Concern. http://www.dpd.cdc.gov/dpdx/HTML/Frames/A-F/Babesiosis/body_Babesiosis_MolDiag.htm. Last modified 7/20/09. Accessed Mar 6, 2011

120. World Health Organization. Guidelines on Standard Operating Procedures for MICROBIOLOGY, Chapter 18 - Malaria. WHO Regional Office for South-East Asia 2010. Available at http://www.searo.who.int/en/Section10/Section17/section 53/section482_1800.htm. Last updated 04/27/2006. Accessed March 17, 2011.

121. Clinical and Laboratory Standards Institute. Laboratory Diagnosis of Blood-borne Parasitic Diseases; Approved Guideline--NCCLS document M15-A. (ISBN 1-56238-401-5). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2000.

122. Warhurst DC, Williams JE. ACP Broadsheet no 148. July 1996. Laboratory diagnosis of malaria. *J Clin Pathol*. 1996;49:533-538.

123. Chisholm ES, Sulzer Ai, Ruebush TK 2d. Indirect immunofluorescence test for human *Babesia microti* infection: antigenic specificity. *Am J Trop Med Hyg.* 1986;35:921-925.

124. Houghton RL, Homer MJ, Reynolds LD, et al. Identification of *Babesia* microti-specific immunodominant epitopes and development of a peptide EIA for detection of antibodies in serum. *Transfusion*. 2002;42:1488-1496.

125. Rosenblatt JE. Laboratory diagnosis of infections due to blood and tissue parasites. *Clin Infect Dis.* 2009;49:1103-1108.

126. Persing DH, Mathiesen D, Marshall WF, et al. Detection of *Babesia microti* by polymerase chain reaction. *J Clin Microbiol*. 1992;30:2097-2103.

127. Reiter I, Weiland G. Recently developed methods for the detection of babesial infections. *Tran R Soc Trop Med Hyg.* 1989;83:21-23.

128. Gubernot DM, Nakhasi HL, Mied PA, Asher DM, Epstein JS, Kumar S. Transfusion-transmitted babesiosis in the United States: summary of a workshop. *Transfusion*. 2009;49:2759-2771.

129. Wormser GP, Dattwyler RJ, Shapiro ED, et al. The clinical assessment, treatment, and prevention of lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis.* 2006;43:1089-1134. Erratum in: *Clin Infect Dis.* 2007;45:941.

130. Iacopino V, Earnhart T. Life-threatening babesiosis in a woman from Wisconsin. *Arch Intern Med.* 1990;150:1527-1528.

131. Shih CM, Wang CC. Ability of azithromycin in combination with quinine for the elimination of babesial infection in humans. *Am J Trop Med Hyg.* 1998;59:509-512.

132. Bonoan JT, Johnson DH, Cunha BA. Life-threatening babesiosis in an asplenic patient treated with exchange transfusion, azithromycin, and atovaquone. *Heart Lung.* 1998;27;424-428.

133. Falagas ME, Klempner MS. Babesiosis in patients with AIDS: a chronic infection presenting as fever of unknown origin. *Clin Infect Dis.* 1996;22:809-812.

134. Raoult D, Soulayrol L, Toga B, Dumon H, Casanova P. Babesiosis, pentamidine, and cotrimoxazole [letter]. *Ann Intern Med.* 1987;107:944.

Acknowledgements

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